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Green tea and skin protection
Mechanism of action and practical applications

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ABSTRACT: Many products currently on the market aim, in one way or another, to apply basic science discoveries obtained using fresh preparations of green tea materials to skin conditions via formulations that of necessity must have a shelf life. However, what is not appreciated fully is the instability of aqueous green tea preparations, and their relative impermeability in skin. This perspective examines some of the difficulties in translating basic science knowledge into efficacious commercial skin products, outlines some potential clinical applications, and describes a recent advance that points a way towards a strategy to improve commercial formulations.

INTRODUCTION

By 2005, basic science research had provided data suggesting green tea (and its chemical contents) could have beneficial effects on the skin and might provide protection against certain adverse conditions. This led to predictions that interest in research and development of skin care products would gather momentum, and new products containing green tea phytochemicals would be introduced to the market faster than ever (e.g., 1). Since then, there have been 87 peer-reviewed articles on the topic of green tea and skin, representing approximately 37 percent of all published scientific articles on this combination of terms, and it becomes more difficult to find skin care products that have no ingredient related to green tea. A Google search for "green tea, skin products" produces 1.55 million links; "makeup, green tea" 1.1 million, "skin care, green tea" 1.5 million, and "green tea, skin protection" 193,000 links. However, although the basic science behind the effects of green tea components has made great strides (albeit primarily in tissue culture and animal studies), the mechanisms underlying the protective effects of green tea on skin remain to be fully elucidated. Moreover, there is a lack of clinical trials confirming efficacy of specific commercial products. Importantly, there are significant practical issues regarding the stability and skin permeability of aqueous green tea extracts that are important in translating basic science research into useful products. Collectively, the science behind the overwhelming number of skin care/skin protection products on the market is not always well-defined. This perspective outlines some of the factors that cause skin damage and skin aging, highlights relevant aspects of our current knowledge of the mechanisms underlying green tea-associated skin protection, and presents selected clinical opportunities for the application of green tea-derived materials to skin conditions where the current treatments have serious adverse side effects. A major purpose is to describe the practical difficulties in developing active green tea-based products, and delineate one strategy-lipid derivatised green tea polyphenols-that has the potential to address these difficulties for future products for skin protection.

The skin is the largest organ of human body. Its functions are well defined: it provides protection against water loss, microbial infection, and environmental elements. When skin is under attack by microbial agents such as viruses or bacteria, it often reacts by local inflammation. Inflammation can also be cause by internal elements such as autoimmune diseases that are associated with damage to the skin, resulting in skin lesions, rash, and altered appearance. A major external element causing skin damage is ultraviolet (UV) light. Both UV-A (315-400 nm) and UV-B (280-315 nm) radiation cause severe skin damage and aging, and are important factors in the etiology of certain types of skin cancer (2). The mechanisms of UV-induced skin damage are mainly *via* local elevation of oxidative load, commonly referred to as oxidative stress, leading to oxidation of lipids and proteins, and free radical-induced DNA damage (2). UV irradiation also alters immune function and induces skin cell death, further complicating the homeostasis of the skin (3). The end result can be local inflammation, cell death (apoptosis), DNA mutation, accelerated skin aging, and skin cancer. It is alarming that the incidence of skin cancer has reached more than a million cases per year in the United States. While cases of most types of cancer are on a decline, according to the National Cancer Institute, those of melanoma (a deadly skin cancer) have tripled during the past 35 years. Adequate protection of the skin from UV irradiation would save many lives and prevent further increases in skin cancer incidence.

Another extrinsic cause of skin damage is infection of skin cells, especially by viruses. Certain viruses, such as herpes simplex virus (HSV) 1 and 2 and human papillomavirus (HPV), can cause skin lesions, pain, and even cancer. A recent estimate indicates that the HSV-2 infected population world wide exceeds 536 million, with 23.6 million new cases each year (4). For HSV-1, it is suggested that more than 70 percent of the Western population is infected (5). Currently, there is no effective strategy to prevent infection with, or recurrence of, HSV infection. Genital human papillomavirus (HPV) is

associated with genital warts, the most commonly occurring sexually transmitted viral infection in humans (6). Newly developed vaccines do appear to prevent an initial infection (7). However, due to the large number of sub-strains of HPV and the lack of long-term efficacy and safety information, with 6.2 million new HPV infections occurring each year, HPV-induced genital warts remains a persistent problem in the human population, especially for women (8).

Finally, autoimmune disorders, the third most common category of disease in the United States, affect about 8 percent of the US population. Among these autoimmune diseases, many are directly related to the skin. Autoimmune diseases are often characterized by their unclear pathogenesis and the lack of effective treatment. A typical example of a cutaneous autoimmune disease is psoriasis, which affects up to 2.2 percent of the U.S. adult population. The pathogenesis of psoriasis is still under investigation. About 5 million Americans suffer from this disease, often with a significant negative impact on their quality of life (National Psoriasis Foundation Benchmark Survey on Psoriasis and Psoriatic Arthritis). Psoriasis involves inflammation, aberrant differentiation, and hyper-proliferation of skin cells, leading to skin lesions and an altered stratum corneum. The pathogenesis of psoriasis involves T-lymphocyte-mediated autoimmune reactions, autoantigen expression, and apoptosis (10). In psoriatic skin, oxidative stress is increased, marked by accumulation of oxidized low density lipoprotein (oxLDL) (11). Current treatment includes photo-therapies, topical steroids, oral retinoids, immunosuppressive agents, and biological agents that block cytokine or cellular immune factors. However, many of these treatment modalities are associated with untoward side effects. For example, a combination of psoralen and UVA, referred to as PUVA therapy, is used for treatment of psoriasis (12). Prolonged treatment with PUVA increases the risk of skin cancer, especially squamous cell carcinoma (12). The recent recall of Raptiva (by Genentech), an immune suppressor for patients with moderate to severe plaque psoriasis, was due to multiple deaths caused by a rare brain viral infection, linked to the medication. These side effects suggest that current approaches to psoriasis can be improved upon. Another cutaneous inflammatory disease, seborrheic dermatitis ("dandruff"), characterized by erythema and/or flaking or scaling in areas of the scalp, affects up to 5 percent of the US population (13). Surprisingly, coal tar, a by-product of coal processing, is still the leading approach for treatment of both psoriasis and seborrehic dermatitis due to the lack of effective alternatives (14). Coal tar is a smelly, messy, staining, carcinogenic material, with short term side effects such as folliculitis, irritation and contact allergy. There have been no clinical studies to evaluate the consequences of long term topical use (14).

During the past five years, scientists have gathered a considerable amount of information regarding the value of green tea-derived compounds for skin protection against aging, UV irradiation-induced damage, and autoimmune diseases. After the discovery by our group that green tea polyphenols (GTPs) can induce human epidermal keratinocyte differentiation (15), we proposed that the topical use of GTPs, or epigallocetachin-3-gallate (EGCG, the most abundant GTP in green tea leaves), could be used to treat various skin conditions (1). In general, application of GTPs could reduce UV-induced erythema, UV-induced sunburn response, DNA damage, elevation of reactive oxygen species (ROS), photo aging of the skin, and modulating of numerous factors related to apoptosis, inflammation, differentiation and carcinogenesis. Specifically, in addition to their ROS-scavenging properties, GTPs prevent UVB-induced skin damage through induction of expression of the immunoregulatory cytokine interleukin (IL)-12, and subsequent IL-12-dependent DNA repair (16). Recently, it was reported that GTPs reduced p53 expression in the skin after UV irradiation, resulting in decreased cell death and damage (17). Data from a knockout mouse study confirmed that caspase 14 expression is essential for skin barrier integrity and photo protection (18). We have demonstrated that EGCG induces signals leading to the expression of caspase 14 and p57 are transmitted *via* the mitogen-activated protein kinase (MAPK) pathways, and importantly, exogenous caspase 14 expression in skin cancer cells significantly reduced the growth of tumours and induced tumour cell death (19). In the deeper portion of the skin, the dermis, collagen can be damaged by UV irradiation through elevation of matrix metalloproteinases (MMP), including collagenases. The destruction of the collagen network in the skin leads to wrinkles and fine lines. It was found that GTPs, and EGCG in particular, inhibit the production of MMPs by modulating the MAPK pathways (20). In summary, the modes of action of GTPs for the photo protection of the skin include ROS scavenging and regulation of genes to inhibit apoptosis, reduce inflammation, improve the skin barrier, and to repair DNA. The central pathways for mediating these responses to GTPs are likely to be the MAPK pathways. It is important to notice that all of these results from the laboratory were generated using freshly-prepared GTPs or EGCG, with maximal activity, and therefore stability was not an issue.

Recently, the antimicrobial properties of GTPs have been recognized (21). Here, we will focus on antiviral activities. Several reports have presented data indicating a strong link between GTPs and prevention of infection by a range of viruses, such as adenovirus (22), Epstein-Barr virus (23), human immunodeficiency virus (HIV) (24), and influenza virus (25). For skin-related viral infections, a study using a cell culture model found that EGCG inactivated HSV (26). This provides hope for novel approaches to prevent HSV infection, and awaits additional studies and clinical trials. Interestingly, clinical studies demonstrated the effectiveness of GTPs in treating genital warts associated with HPV (27). These clinical trials led to a new FDA-approved prescription drug, Veregen, for genital wart treatment. This topical

medication (an ointment containing 15 percent of a defined green tea leaf extract called Polyphenon E or sinecatechins) represents the first FDA-approved "drug" with Polyphenon E/ sinecatechins as an active ingredient (although green tea was originally used as an ingested medication two thousand years ago, and later evolved to a beverage). It has been proposed that the antiviral properties of GTPs are due to their powerful protein binding capacity, resulting in tight binding to the viral coat proteins, and to their ability to modulate the dynamics of the cell plasma membrane, thereby preventing the entry of viral particles into the cells. However, the exact mechanisms are yet to be identified.

For the skin manifestations of autoimmune diseases, several mechanisms underlying the protective effects of GTPs have been identified. We showed that, in a mouse model for human psoriasis (the flaky skin mouse), topical application of freshly-prepared 0.5 percent GTPs prevented psoriasiform lesions and improved skin histology (28). The protective role of GTPs is associated with caspase 14 expression and processing to the active form, and suppression of hyperproliferation, with increased differentiation (28).
In addition, cytokine production is another major contributor to autoimmune diseases. Evidence from many studies shows that GTPs may moderate or prevent diseases associated with cytokine over expression (29). Consistent with this, GTPs potently inhibit production of TNF-a (a pro-apoptotic cytokine) in the macrophage cell line RAW264.7, and in elicited mouse peritoneal macrophages. Further, EGCG inhibits TNF-a-mediated activation of the nuclear factor-κB (NF-κB) pathway, thereby protecting normal cultured epithelial cells from TNF-a-induced apoptosis. Another inflammatory cytokine, IL-1, increases the production and activity of matrix metalloproteinases (MMPs). EGCG effectively inhibited IL-1β-induction of MMP-1 and MMP-13 (reviewed in ref 30). Significantly, the inhibition of IL-1β signalling may be through modulation of the MAPK signalling pathway components. These effects of GTPs/EGCG have been confirmed in various animal models for autoimmune diseases (30). Taken together, these data show that GTPs can reduce the severity of autoimmune diseases despite the likelihood of different initial triggers of each disease. In summary, the beneficial effects of GTPs on autoimmune diseases are associated with inhibition of hyper proliferation, suppression of cytokine over-expression, and their well-known antioxidant activity, and these effects are mediated in part by the MAPK pathways.

It is clear that the unique chemical composition of green tea makes it, in principle, an ideal ingredient for topical application against UV-induced skin damage, viral infection, and autoimmune-induced skin disorders. What has prevented the more wide spread use of GTPs in medications for treating these conditions? Why is there to date only one FDA-approved drug that has GTPs as an active ingredient? Besides the lack of well-controlled clinical studies, the chemical nature of these compounds is a major negative factor for the development of topical medications. The initial excitement over the non-toxic, natural skin medication Veregen did not last for this reason. It failed to become a popular topical medication to treat genital warts not because GTPs don't work; it worked well in multiple clinical trials. Unfortunately, GTPs are unstable, and the hydrophilic nature of those compounds requires a high dose for an effect on the skin, a water-proof barrier. Similar to bottled green tea drinks, the GTPs in these preparations are constantly undergoing oxidation and polymerization, and therefore the chemical composition cannot be kept stable in any such preparation. A simple test demonstrates the instability of green tea compounds: a cup of brewed green tea will turn dark if it is put at room temperature overnight. This instability is compounded with the undesirable effect of an extremely high concentration of GTPs (15 percent): unsightly skin staining (17). Certainly, GTPs in their original forms are not likely to be "active ingredients" in any skin products unless they are freshly prepared or their activity is stabilized.

In 2005, we identified two major challenges for the formulation of GTPs into skin care products or topical medications for the skin: their instability and low bioavailability. We stated then that "changing the physical properties of GTPs by molecular modification to increase the stability and improve skin penetration may be an option" (1). Today, these challenges have potentially been overcome with the invention of modified GTPs, referred to as lipophilic tea polyphenols (LTP) (31). The modification of GTPs is the ester linkage of fatty acid molecules to the hydroxyl groups of GTPs, resulting in a mixture of highly stable GTPs molecules protected by the ester groups. In addition, purified EGCG esters have been made either by a chemical method, or an enzymatic method (32). The availability of these new materials now makes it possible to develop novel topical applications for skin care and skin protection, as well as topical medications for the treatment of various skin conditions.

Our preliminary results have shown that LTP are able to induce caspase 14 expression in normal human epidermal keratinocytes (NHEK). When LTP entered NHEK, EGCG was released into the cytoplasm by esterases (31). The time of release could potentially be controlled by using different fatty acids to form esters with GTPs with different hydrolysis rates. Very few skin care products using this technology are currently available on the market, but importantly, those on the market receive the highest rating by users when compared to peer products. Further encouraging evidence for the antiviral properties of LTP is that EGCG-palmitate, a palmitic acid ester of EGCG, is 24 times stronger than EGCG against influenza virus infection (31). These emerging results, although preliminary, hint at a breakthrough in the near future in the therapeutic and cosmetic use of green tea-derived phytochemicals, and a new era in the treatment of epidermal diseases and skin protection.

With supporting evidence obtained from clinical studies using GTPs, such as the human study by Elmet et al. (33), clinical trials using LTP-containing formulations are warranted. We would not be surprised to see, in a few years, a large number of products, with adequate clinical study support, for uses such as sunscreen, sun repair, HSV prevention, HPV prevention, genital wart treatment, influenza prevention, anti-aging, psoriasis treatment, and anti-dandruff shampoo.

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